This Listing of Claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

Claim 1 (currently amended): A purified~~, large-scale~~ preparation comprising ~~at least 200 grams of~~ tissue factor pathway inhibitor (TFPI) or TFPI analog molecules, wherein less than ~~12%~~ 2% of the TFPI or TFPI analog molecules are ~~modified species, wherein the modified species include one or more of the following:~~

~~an oxidized TFPI or TFPI analog molecule, as detected by reverse phase chromatography;~~

a carbamylated TFPI or TFPI analog molecule, as detected by cation exchange chromatography~~;~~

~~a deamidated TFPI or TFPI analog molecule, as detected through indirect measurement of isoaspartic acid;~~

~~a TFPI or TFPI analog molecule that comprises a cysteine adduct, as determined by amino acid analysis;~~

~~aggregated TFPI or TFPI analog molecules, as detected by size exclusion chromatography; and~~

~~a misfolded TFPI or TFPI analog molecule, as detected by non-denaturing SDS-polyacrylamide gel electrophoresis~~.

Claim 2 (canceled)

Claim 3 (currently amended): The purified preparation of claim 1, wherein less than about ~~3%~~ 1% of the TFPI or TFPI analog molecules are carbamylated.

Claims 4-7 (canceled)

Claim 8 (currently amended): The purified preparation of claim 1, wherein ~~members of~~ the ~~plurality of~~ TFPI molecules have the amino acid sequence shown in SEQ ID NO:1.

Claim 9 (previously presented): The purified preparation of claim 1, wherein the TFPI analog molecules are ala-TFPI molecules.

Claim 10 (currently amended): A ~~large-scale~~ pharmaceutical formulation comprising ~~at least 200 grams of tissue~~ factor pathway inhibitor (TFPI) or TFPI analog molecules, wherein less than ~~12%~~ 2% of the TFPI or TFPI analog molecules are ~~modified species wherein the modified species include one or more of the following:~~

~~an oxidized TFPI or TFPI analog molecule, as detected by reverse phase chromatography;~~

a carbamylated TFPI or TFPI analog molecule, as detected by cation exchange chromatography~~;~~

~~a deamidated TFPI or TFPI analog molecule, as detected through indirect measurement of isoaspartic acid;~~

~~a TFPI or TFPI analog molecule that comprises a cysteine adduct, as determined by amino acid analysis;~~

~~aggregated TFPI or TFPI analog molecules, as detected by size exclusion chromatography; and~~

~~a misfolded TFPI or TFPI analog molecule, as detected by non-denaturing SDS-polyacrylamide gel electrophoresis~~.

Claim 11 (canceled)

Claim 12 (currently amended): The pharmaceutical formulation of claim 10, wherein less than about ~~3%~~ 1% of the TFPI or TFPI analog molecules are carbamylated.

Claims 13-16 (canceled)

Claim 17 (currently amended): The pharmaceutical formulation of claim 10, wherein ~~members of the plurality of TFPI or TFPI analog molecules are~~ TFPI molecules ~~that~~ have the amino acid sequence shown in SEQ ID NO:1.

Claim 18 (currently amended): The pharmaceutical formulation of claim 10, wherein ~~members of the plurality of TFPI or~~ TFPI analog molecules are ala-TFPI molecules.

Claim 19 (currently amended): A ~~large-scale~~ pharmaceutical formulation comprising:

~~at least 200 grams of~~ tissue factor pathway inhibitor molecules having an additional amino terminal alanine residue (ala-TFPI), wherein less than ~~12%~~ 2% of the TFPI or TFPI analog molecules are ~~modified species, wherein the modified species include one or more of the following:~~

~~an oxidized ala-TFPI molecule, as detected by reverse phase chromatography;~~

a carbamylated ala-TFPI molecule, as detected by cation exchange chromatography~~;~~

~~a deamidated ala-TFPI molecule, as detected through indirect measurement of isoaspartic acid;~~

~~an ala-TFPI molecule that comprises a cysteine adduct, as determined by amino acid analysis;~~

~~aggregated TFPI or TFPI analog molecules, as detected by size exclusion chromatography; and~~

~~a misfolded ala-TFPI molecule, as detected by non-denaturing SDS-polyacrylamide gel electrophoresis~~,

wherein the pharmaceutical formulation comprises 20 mM sodium citrate, 300 mM L-arginine, and 5 mM methionine, pH 5.5.

Claim 20 (withdrawn-currently amended): A method of producing the purified~~, large-scale~~ preparation of claim 1, comprising the steps of:

(1) expressing TFPI or a TFPI analog in a rifampicin-resistant *E. coli* host cell, wherein the TFPI or the TFPI analog is encoded on a plasmid comprising the following elements:

(a) a transcription promoter;

(b) a ribosome binding site adjacent to the reclac transcription promoter;

(c) a nucleotide coding sequence that encodes the TFPI or the TFPI analog adjacent to the ribosome binding site;

(d) a transcription terminator adjacent to the nucleotide coding sequence;

(e) a replicon;

(f) an antibiotic resistance gene; and

(g) a gene encoding an N-terminal methionine-removing enzyme;

(2) isolating inclusion bodies containing the TFPI or the TFPI analog from the *E. coli* host cell;

(3) isolating the TFPI or the TFPI analog from the inclusion bodies to obtain isolated TFPI or TFPI analog;

(4) refolding the isolated TFPI or TFPI analog to form refolded TFPI or TFPI analog;

(5) purifying the refolded TFPI or TFPI analog by SP-Sepharose fast flow chromatography in the presence of Mg^{++} to form a first preparation of purified TFPI or TFPI analog;

(6) concentrating the first preparation of purified TFPI or TFPI analog to form a first concentrated preparation of purified TFPI or TFPI analog;

(7) purifying the first concentrated preparation of purified TFPI or TFPI analog by Q-Sepharose HP chromatography to form a second preparation of purified TFPI or TFPI analog;

(8) purifying the second preparation of purified TFPI or TFPI analog by butyl HIC chromatography to form a third preparation of purified TFPI or TFPI analog;

(9) purifying the third preparation of purified TFPI or TFPI analog by SP-Sepharose HP chromatography to form a fourth preparation of purified TFPI or TFPI analog;

(10) concentrating the fourth preparation of purified TFPI or TFPI analog to form a second concentrated preparation of purified TFPI or TFPI analog molecules~~, wherein less than about 12% of the TFPI or TFPI analog molecules are modified TFPI or TFPI analog molecules~~.

Claim 21 (withdrawn): The method of claim 20 wherein the transcription promoter is a reclac promoter.

Claim 22 (withdrawn): The method of claim 20 wherein the ribosome binding site is the ribosome binding site from gene 10 of bacteriophage T7.

Claim 23 (withdrawn): The method of claim 20 wherein the nucleotide coding sequence encodes ala-TFPI.

Claim 24 (withdrawn): The method of claim 23 wherein the nucleotide coding sequence is SEQ ID NO:44.

Claim 25 (withdrawn): The method of claim 20 wherein the transcription terminator comprises the nucleotide sequence shown in SEQ ID NO:42.

Claim 26 (withdrawn): The method of claim 20 wherein the replicon comprises a pBR322 origin of replication.

Claim 27 (withdrawn): The method of claim 20 wherein the replicon comprises a rop copy number control gene from pBR322.

Claim 28 (withdrawn): The method of claim 20 wherein the antibiotic resistance gene is streptomycin adenyltransferase.

Claim 29 (withdrawn): The method of claim 20 wherein the N-terminal methionine-removing enzyme is *E. coli* methionine aminopeptidase.

Claim 30 (withdrawn): The method of claim 20 wherein the *E. coli* host cell is MON210 (ATCC Accession No. PTA-5564).

Claim 31 (withdrawn-currently amended): A method of purifying tissue factor pathway inhibitor (TFPI) or TFPI analog molecules to provide the purified~~, large-scale~~ preparation of claim 1, comprising the steps of:

(1) purifying recombinantly produced TFPI or TFPI analog molecules by SP-Sepharose fast flow chromatography to form a first preparation of purified TFPI or TFPI analog;

(2) concentrating the first preparation of purified TFPI or TFPI analog to form a first concentrated preparation of purified TFPI or TFPI analog;

(3) purifying the first concentrated preparation of purified TFPI or TFPI analog by Q-Sepharose HP chromatography to form a second preparation of purified TFPI or TFPI analog;

(4) purifying the second preparation of purified TFPI or TFPI analog by butyl HIC chromatography to form a third preparation of purified TFPI or TFPI analog;

(5) purifying the third preparation of purified TFPI or TFPI analog by SP-Sepharose HP chromatography to form a fourth preparation of purified TFPI or TFPI analog;

(6) concentrating the fourth preparation of purified TFPI or TFPI analog to form a second concentrated preparation of purified TFPI or TFPI analog molecules~~, wherein less than about 12% of the TFPI or TFPI analog molecules are modified TFPI or TFPI analog molecules~~.

Claim 32 (withdrawn): The method of claim 31 wherein the SP-Sepharose fast flow chromatography is performed in the presence of Mg^{++}.

Claim 33 (withdrawn): The method of claim 31 wherein the TFPI or TFPI analog molecules are produced in yeast cells.

Claim 34 (withdrawn): The method of claim 31 wherein the TFPI or TFPI analog molecules are produced in mammalian cells.

Claim 35 (withdrawn): The method of claim 34 wherein the mammalian cells are CHO cells.

Claim 36 (withdrawn): The method of claim 34 wherein the mammalian cells are HepG2 cells.

Claim 37 (withdrawn): The method of claim 34 wherein the mammalian cells are Chang liver cells.

Claim 38 (withdrawn): The method of claim 34 wherein the mammalian cells are SK hepatoma cells.

Claim 39 (withdrawn-currently amended): A method of expressing tissue factor pathway inhibitor (TFPI) or TFPI analog to provide the purified~~, large-scale~~ preparation of claim 1, comprising:

(1) culturing a rifampicin-resistant *E. coli* host cell in a fermentation medium, wherein the *E. coli* host cell comprises a plasmid having the following elements:

(a) a transcription promoter;

(b) a ribosome binding site adjacent to the reclac transcription promoter;

(c) a nucleotide coding sequence that encodes TFPI or TFPI analog adjacent to the ribosome binding site;

(d) a transcription terminator adjacent to the nucleotide coding sequence;

(e) a replicon;

(f) an antibiotic resistance gene; and

(g) a gene encoding an N-terminal methionine-removing enzyme;

wherein one liter of the fermentation medium comprises 41 g dextrose, 2.5 g $(NH_4)_2SO_4$, 4.0 g sodium polyphosphate, 7.0 g K_2SO_4, 1.63 g $MgSO_4 \cdot 7H_2O$, 2.0 g methionine, 2.0 g glycerol, 0.5 mg H_3BO_4, 0.5 g cobalt chloride, 0.13 g $CuSO_4 \cdot 6H_2O$, 54.0 g $FeCl_3 \cdot 6H_2O$, 11.0 g $MnSO_4 \cdot H_2O$, 0.5 g $Na_2MoO_4 \cdot 2H_2O$, 0.02 $NaSeO_3$, 22.0 g $ZnSO_4 \cdot 7H_2O$, 0.01 ml concentrated H_2SO_4, and 0.55 ml UCON antifoam.

Claim 40 (withdrawn): The method of claim 39 wherein the transcription promoter is a reclac promoter.

Claim 41 (withdrawn): The method of claim 39 wherein the ribosome binding site is the ribosome binding site from gene 10 of bacteriophage T7.

Claim 42 (withdrawn): The method of claim 39 wherein the nucleotide coding sequence encodes ala-TFPI.

Claim 43 (withdrawn): The method of claim 42 wherein the nucleotide coding sequence is SEQ ID NO:44.

Claim 44 (withdrawn): The method of claim 39 wherein the transcription terminator comprises the nucleotide sequence shown in SEQ ID NO:42.

Claim 45 (withdrawn): The method of claim 39 wherein the replicon comprises a pBR322 origin of replication.

Claim 46 (withdrawn): The method of claim 39 wherein the replicon comprises a rop copy number control gene from pBR322.

Claim 47 (withdrawn): The method of claim 39 wherein the antibiotic resistance gene is streptomycin adenyltransferase.

Claim 48 (withdrawn): The method of claim 39 wherein the N-terminal methionine-removing enzyme is *E. coli* methionine aminopeptidase.

Claim 49 (withdrawn): The method of claim 39 wherein the *E. coli* host cell is MON210 (ATCC Accession No. PTA-5564).

Claim 50 (currently amended): The purified~~, large-scale~~ preparation of claim 1 comprising 200 grams to 2.4 kilograms of tissue factor pathway inhibitor (TFPI) or TFPI analog.

Claim 51 (currently amended): The purified~~, large-scale~~ preparation of claim 50 comprising 200-300 grams of tissue factor pathway inhibitor (TFPI) or TFPI analog.

Claim 52 (currently amended): The purified~~, large-scale~~ preparation of claim 50 comprising 400-600 grams of tissue factor pathway inhibitor (TFPI) or TFPI analog.

Claim 53 (currently amended): The purified~~, large-scale~~ preparation of claim 50 comprising 600-900 grams of tissue factor pathway inhibitor (TFPI) or TFPI analog.

Claim 54 (currently amended): The purified~~, large-scale~~ preparation of claim 50 comprising 800-1200 grams of tissue factor pathway inhibitor (TFPI) or TFPI analog.

Claims 55-57 (canceled)

Claim 58 (new): A method of preparing a pharmaceutical composition comprising tissue factor pathway inhibitor (TFPI) or ala-TFPI molecules, wherein less than 2% of the TFPI or ala-TFPI molecules are carbamylated molecules, as detected by cation exchange chromatography, the method comprising:

(a) purifying refolded TFPI or ala-TFPI, which has been isolated from inclusion bodies following expression in a host cell, with a sequence of chromatography operations to provide a purified, refolded TFPI or ala-TFPI preparation,

(b) concentrating and diafiltering the purified, refolded TFPI or ala-TFPI preparation to provide a TFPI or ala-TFPI drug substance, and

(c) formulating the TFPI or ala-TFPI drug substance into the pharmaceutical composition,

wherein the sequence of chromatography operations comprises two cation exchange chromatography operations, an anion exchange chromatography operation, and a hydrophobic interaction chromatography operation.

Claim 59 (new): The method of claim 58, wherein the sequence of chromatography operations comprises, in order, a first cation exchange chromatography operation, an anion exchange chromatography operation, a hydrophobic interaction chromatography operation, and a second cation exchange chromatography operation.

Claim 60 (new): The method of claim 59, wherein the first cation exchange operation is performed in the presence of urea.

Claim 61 (new): The method of claim 59, wherein the first cation exchange operation is performed using a sodium citrate elution buffer.

Claim 62 (new): The method of claim 58, wherein the TFPI or ala-TFPI drug substance has a protein concentration of about 10 mg/ml.

Claim 63 (new): The method of claim 58, wherein the pharmaceutical composition contains 0.15 mg/ml ala-TFPI, 20 mM sodium citrate, 300 mM L-arginine, and 5 mM methionine and has a pH of 5.5.

Claim 64 (new): The method of claim 58, wherein, in step (b), the purified, refolded TFPI or ala-TFPI preparation is concentrated and diafiltered into a buffer to provide the TFPI or ala-TFPI drug substance.

Claim 65 (new): The method of claim 64, wherein the buffer comprises 300 mM L-arginine and 20 mM sodium citrate and has a pH of 5.5.

Claim 66 (new): The method of claim 64, wherein the TFPI or ala-TFPI drug substance is storage stable at < 60°C for at least 24 months.

Claim 67 (new): The method of claim 58, wherein step (a) comprises:

(a1) purifying the refolded TFPI or ala-TFPI by SP-Sepharose fast flow chromatography to form a first preparation of purified TFPI or ala-TFPI,

(a2) concentrating the first preparation of purified TFPI or ala-TFPI to form a first concentrated preparation of purified TFPI or ala-TFPI,

(a3) purifying the first concentrated preparation of purified TFPI or ala-TFPI by Q-Sepharose HP chromatography to form a second preparation of purified TFPI or ala-TFPI,

(a4) purifying the second preparation of purified TFPI or ala-TFPI by butyl HIC chromatography to form a third preparation of purified TFPI or ala-TFPI, and

(a5) purifying the third preparation of purified TFPI or ala-TFPI by SP-Sepharose HP chromatography to provide the purified, refolded TFPI or ala-TFPI preparation.

Claim 68 (new): The method of claim 67 wherein the SP-Sepharose fast flow chromatography is performed in the presence of Mg^{++}.

Claim 69 (new): The method of claim 58, wherein step (a) provides the purified, refolded TFPI or ala-TFPI preparation in an amount from 200 grams to 2.4 kilograms.

Claim 70 (new): The method of claim 58, further comprising, prior to step (a),

(1) expressing the TFPI or ala-TFPI in an *E. coli* host cell,

(2) isolating inclusion bodies containing the TFPI or ala-TFPI from the *E. coli* host cell,

(3) isolating the TFPI or ala-TFPI from the inclusion bodies to obtain isolated TFPI or ala-TFPI, and

(4) refolding the isolated TFPI or ala-TFPI to provide the refolded TFPI or ala-TFPI.

Claim 71 (new): The method of claim 70, wherein the *E. coli* host cell is rifampicin-resistant.

Claim 72 (new): The method of claim 70, wherein the *E. coli* host cell comprises a plasmid comprising:

(a) a reclac transcription promoter,

(b) a nucleotide coding sequence that encodes the TFPI or ala-TFPI,

(c) a transcription terminator adjacent to the nucleotide coding sequence, and

(d) a plasmid copy number control rop gene.

Claim 73 (new): The method of claim 72, wherein the plasmid further comprises an antibiotic resistance gene.

Claim 74 (new): The method of claim 73, wherein the antibiotic resistance gene is an aminoglycoside nucleotidyltransferase gene that confers resistance to streptomycin and spectinomycin.

Claim 75 (new): The method of claim 72, wherein the plasmid further comprises a gene encoding an N-terminal methionine-removing enzyme.